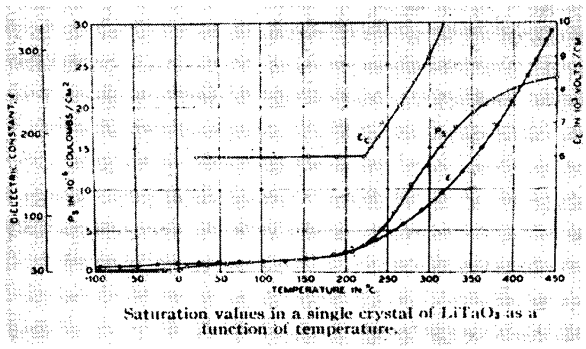

FIG. 12. Dielectric behavior of a $LiTaO_3$ crystal.

rhombohedral metatitanates before any conclusions as to their dielectric behavior could be reached.

We have tried to show that all metal-oxygen octahedra which fulfill certain conditions will always cause very high polarizabilities and are therefore the cause of ferroelectricity in those compounds that do not contain hydrogen. Other octahedra which do not satisfy the conditions may give rise to many different kinds of transitions, but never give high dielectric constants or ferroelectricity (*32*). The theoretical aspects concerning these octahedra have been and will be published by Jaynes and Wigner (*28*). The recent paper by Slater (*26*) on the correction of the Lorentz factor in barium titanate confines itself to this compound. Slater comes to the conclusion that the TiO strings running through the lattice are the essential factor for the occurrence of ferroelectricity.

Lower Curie point in $LiTaO_3$. Except for Rochelle salt, there never had been observed a lower Curie point—that is, a temperature *below* which the spontaneous polarization disappears completely. Only recently in $LiTaO_3$ a sharp decrease of the spontaneous polarization with decreasing temperature has been measured (*9*). A numerical treatment will be able to decide whether this is due to the stronger interaction between neighboring octahedra on account of their closer contact with each other. In the ilmenite structure neighboring octahedra share corners *and* edges, whereas in the perovskite system the octahedra only share corners.

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Technical Papers

The Mucopolysaccharides of the Ground Substance of Connective Tissue[1]

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The dramatic effects of cortisone and adrenocorticotrophic hormone (ACTH) on the manifestations of rheumatic diseases, as well as on some not classified as rheumatic, have forcefully reemphasized the importance of the connective tissues in the mechanism of a variety of disorders. It seems remarkable that we still know so little about the biochemistry and physiology of the mesenchyma, even though its contribution to biological processes and its paramount importance in pathology had been recognized long before the advent of cortisone.

In the past 20 years, the ground substance(s) of the mesenchyma have been studied extensively. The reawakened interest in these tissues was due in part to the isolation and characterization of some of the

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component mucopolysaccharides of the ground substance(s) (*1*), and in part to the demonstration of the presence of enzymes in microorganisms and in mammalian tissues which hydrolyze some of these mucopolysaccharides, especially hyaluronic acid (*2*), and to the subsequent identification of these enzymes with the spreading agents (*3*).

The relative simplicity of our earlier concepts of the nature of the complexes presumed to be present in the ground substance(s) is no longer tenable and has had to be revised frequently on the basis of new experimental findings.

During the past few years we have been studying the distribution of the mucopolysaccharides in the skin, heart valves,[2] aorta, tendon,[2] synovial fluid, and umbilical cord. It was not possible with most of these tissues to utilize extraction with neutral salts; such methods do not extract mucopolysaccharide from tendon, aorta, or heart valves. Half-saturated lime water, which has been used extensively in earlier biochemical studies (*4*), and is still used in histological techniques, yields mucopolysaccharides bound to protein in denatured form—i.e., denatured acid mucoids (for nomenclature see [*5*]). We therefore resorted to extraction with 0.33–0.5 *N* NaOH, which hydrolyzes the bonds between carbohydrate and protein. The tissues were extracted at 0°, the extracts then being neutralized with acetic acid. The mucopolysaccharides solubilized by this method were freed of accompanying protein by treatment with amyl alcohol–chloroform, adsorption on Lloyd's reagent, or zinc hydroxide. Glycogen, which is always present in the extracts, was removed by digestion with a commercial amylase. The remaining high molecular polysaccharides were fractionally precipitated from calcium acetate–acetic acid solution by alcohol at 0°. These purification procedures were repeated when the fractions were found to be still contaminated. The fractions were analyzed for nitrogen, hexosamine, uronic acid, and sulfate, and the optical rotation was determined. The hydrolysis of the substances was determined by measuring the increase in reducing sugar on incubation with testicular and, in some instances, with pneumococcal hyaluronidase. The amino sugar of each class of mucopolysaccharide was isolated after acid hydrolysis as the crystalline hydrochloride, and its nature ascertained by analysis and measurement of the optical rotation.

On the basis of this work, we can at present distinguish 5 mucopolysaccharides in connective tissues: (1) Hyaluronic acid, which is sulfate-free, has a specific rotation of $-70°$ to $-80°$, and is digested at a rapid rate by both testicular and pneumococcal hyaluronidase. (2) Chondroitin sulfate A, which has been found only in hyaline cartilage, has a specific rotation in neutral solution of $-30°$, and is hydrolyzed by testicular but not by pneumococcal hyaluronidase. (3) Chondroitin sulfate B, which has the same composition as A, a specific rotation of $-50°$, and is resistant to both testicular and pneumococcal hyaluronidase. On fractionation in calcium acetate solution by means of alcohol, it precipitates at an alcohol concentration of 20% (by volume). This fraction was first isolated from pigskin in 1941 (*6*). (4) Chondroitin sulfate C, which also has the same composition as A, but a specific rotation of $-20°$, and is hydrolyzed by testicular hyaluronidase at a rate that may be faster than that of ChS-A. It precipitates at an alcohol concentration of 50%. (5) Hyaluronosulfate, which has been obtained only from cornea (*7*). It has a specific rotation of $-56°$ and, like hyaluronic acid, is hydrolyzed by both testicular and pneumococcal hyaluronidase. Its amino sugar was found to be D-glucosamine in contrast to that of ChS-A, -B, and -C, from which D-galactosamine was obtained.

[2] C. Ragan and E. E. Fischel collaborated on part of the work on tendon and heart valves, respectively.

TABLE 1

OCCURRENCE OF MUCOPOLYSACCHARIDES IN MESENCHYMAL TISSUES

Group	Tissue	Hyaluronic acid	ChS-A $[\alpha]_D = -30°$	ChS-B $[\alpha]_D = -50°$	ChS-C $[\alpha]_D = -20°$
I	Vitreous humor	+	−	−	−
	Synovial fluid	+	−	−	−
	Mesothelioma	+	−	−	−
II	Hyaline cartilage	−	+	−	+ (?)
III	Heart valves	−	−	+	+
	Tendon (pig and calf)	±	−	+	+
	Aorta	−	−	+	+
IV	Skin (pig and calf)	+	−	+	−
	Umbilical cord	+	−	−	+

Heparin may be mentioned here as a possible sixth mucopolysaccharide occurring in the ground substance(s). Thus far we have not encountered heparin in identifiable concentrations, either because its concentration is too low or because this fraction may be lost in our isolation procedure.

We have summarized the sources of the mucopolysaccharides *based on isolation* in Table 1.

It can be seen that hyaluronic acid occurs in the absence of demonstrable quantities of sulfate esters in vitreous humor, synovial fluid (obtained both from cattle and from patients with rheumatoid arthritis), and the peritoneal fluid of a patient with a mesothelioma.

No hyaluronic acid is found in hyaline cartilage. The low sulfate values of some preparations of ChS-A prepared by this laboratory (*7, 8*), as well as by other workers (*9*), are due to contamination with glycogen. The molar sulfate ratio approximates unity in our experience if subfractions are treated with commercial amylase. Solutions of (protein and glycogen-free) chondroitin sulfate on alcohol fractionation yield 2 fractions: a major fraction of a specific rotation of $-30°$ separating in fibrous form at low

alcohol concentrations, and a minor fraction of a specific rotation of $-20°$ to $-25°$ precipitating in flocculent form at higher alcohol concentration. Whether this fraction is identical with ChS-C of the fibrous connective tissues of Group III cannot be decided at present.

The most significant finding presented in Table 1 is the absence of hyaluronic acid from the 3 connective tissues of Group III—heart valves, tendon, and aorta. In all 3 tissues we found 2 sulfated mucopolysaccharides—ChS-B and ChS-C—in approximately equal quantities. In only one out of five isolation experiments performed on tendon was a small amount of hyaluronic acid found. We believe that this hyaluronic acid was derived from tendon sheaths that were present in this particular batch of tendon.

In contrast to the 3 tissues of Group III, skin and umbilical cord contain hyaluronic acid together with only 1 sulfate ester. In skin this sulfate ester is ChS-B, whereas in umbilical cord it is ChS-C. From both pig- and calfskin, no fraction corresponding to ChS-C could be isolated, and in umbilical cord ChS-B could not be detected. The quantities of both hyaluronate and ChS-C present in this embryonal tissue are many times larger than those found in normal adult tissues.

It may be mentioned here that human skin, both normal and pathological, has similarly been reported to contain hyaluronic acid, together with a sulfate ester presumed to be chondroitin sulfate (*10*).

In addition to strictly chemical problems, the observations reported raise many biological questions, among which may be mentioned the mechanism of formation and the anatomical locations of the various fractions, and their changes under the influences of physiological and pathological stimuli.

Histological techniques such as staining with metachromatic dyes and uptake of acidic and basic dyes at controlled pH values, or staining with the Hotchkiss method in combination with treatment of the histological sections with enzymes, have called attention to the accumulation in various diseases and in experimental conditions of material thought to be mucopolysaccharide in nature, such as in the nodules of rheumatoid arthritis (*11*), in the Aschoff bodies of rheumatic fever and in myocarditis in acute rheumatic carditis (*12*), in lupus erythematosus disseminatus (*13*), in myxedema, in granulation tissue (*14, 15*), in the skin of experimental mammals, and in the coxcomb on application of sex hormones (*16*) or pituitary hormones (*17*). In granulation tissue the quantity of the metachromatic material greatly diminishes as the scar matures (*12, 15*). In human skin the formation of dense collagen fibers is reported to be accompanied by a relative drop of the ratio of hyaluronic acid to chondroitin sulfate (*18*). Metachromatic material surrounds the elastic membrane of the smaller arteries, especially in the intima. In the larger elastic arteries like the aorta and its branches, much metachromatic material is found in the intima (*12*). It has been further pointed out that elsewhere elastic fibers are commonly associated with metachromatic material (*12*).

Other authors (*19*) postulate, on the basis of staining by the Hotchkiss-McManus technique, the presence of glycoproteins as essential components of the basement membranes of skin and of other organs. This basement membrane is said to be absent from embryonal skin but to increase in thickness with aging in skin and in the walls of blood vessels. Recapitulation of these observations may suffice to focus attention on the need for integration of histological data with chemical findings. From the scanty data available it appears that aging and pathological processes, rather than consisting merely in quantitative alterations, involve qualitative changes in the type of mucopolysaccharides present.[3]

Aside from questions of structure and anatomy of the ground substance, the problem of its physiological functions has been almost completely neglected. Although hyaluronic acid occurs in greatly hydrated gels and therefore may be involved in water binding (*21*), a similar function is not apparent for the sulfated mucopolysaccharides and their protein complexes. One function, however, suggests itself for these compounds—that is, their possible role as cationic exchange resins. The dissociation of the carboxyl groups in these compounds is suppressed by the much more strongly dissociated sulfate groups, and is so weak that it seems plausible to assume the ready formation of lactones under the influence of hydrogen ions and the subsequent opening of the lactone rings by hydroxyl ions. The anatomical location of such compounds in the basal membranes as suggested by Gersh and Catchpole would explain the orientation which has to be postulated for a system that has to function biologically.[4]

Experimental studies on the physiological role of the mesenchymal ground substances, and their response to regulation by hormones and to the action of the unknown noxious agents which cause the mesenchymal diseases, depend on the correlation of information obtained by chemical isolation, histological methods, and on studies of cellular metabolism. In such studies histology will play a predominant part, especially as methods are developed that can give more specific and quantitative information than those now in use.

[3] In this paper attention is concentrated on the mucopolysaccharides as components of the ground substance(s). We do not imply that this material is only, or even mainly, composed of polysaccharides. Thus far, we know almost nothing of the protein complexes as they occur in the ground substance except that the protein is not only firmly bound to the sulfated mucopolysaccharides but that it differs from collagen. From histological data it is known that proteolytic enzymes cause dissolution of the ground substance(s) at a rate faster than that effected by hyaluronidases (*19, 20*).

[4] It might be pointed out here that the mucoitin sulfate of the gastric mucosa (*22*) may play a similar catalytic role in the acid production or in the Na^+ shift of this organ.

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Tissue Respiration and Body Size[1]

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The relation between tissue respiration and body size has only recently attracted attention, though it is crucial for a classic and major topic of physiology. This relation is decisive for answering the question of whether the systematic decrease of metabolic rate per unit weight with increasing size, which is found in most animal classes and expressed by the so-called surface law and similar formulations, is based upon cellular or organismic factors. After earlier work of Terroine (*1*), Grafe *et al.* (*2, 3*), Kayser *et al.* (*4*), which was inconclusive and contradictory, Kleiber (*5, 6*) investigated the Q_{O_2} of liver from rats, rabbits, and sheep, and Weymouth *et al.* (*7*) the Q_{O_2} of the midgut gland of individuals of different size in the kelp crab *Pugettia*. These workers state a systematic decrease of the Q_{O_2} values with increasing size, in approximately the same extent as basal metabolism of the entire animal decreases. In a recent investigation, Krebs (*8*) compared the Q_{O_2} of 5 tissues of 9 different species. He found that, in general, Q_{O_2} values of the larger species are somewhat lower than the homologous values of small species; but there is no parallelism of this decrease in the different tissues, nor a consistent relation to the decrease of basal metabolic rate per unit weight with increasing size. There seems to be, as yet, no systematic investigation on the intraspecific size dependence of Q_{O_2} of different tissues.

In our experiments, albino rats (Wistar strain) were used, representing a continuous series from newborn animals of 9 g body weight to adults of 392 g.

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TABLE 1

TISSUE METABOLISM AND BODY SIZE IN THE ALBINO RAT

	No. of experiments	Mean Q_{O_2}	Mean Q_{O_2} for body wt 0–50	50–150	150–250	250 +
Heart	33	9.09 ± .49	10.68	8.48	8.45	7.93
Lung	35	7.15 ± .25	7.90	6.50	6.93	6.34
Liver	49	8.62 ± .22	9.03	9.08	7.20	7.55
Kidney	48	15.50 ± .36	15.70	14.19	16.42	16.85
Brain cortex	30	8.62 ± .25	8.25	8.70	8.35	9.92
Thymus	32	8.25 ± .33	9.04	8.75	7.06	6.99
Diaphragm	26	6.26 ± .51	8.99	6.08	4.75	4.87

The Q_{O_2} ($\mu l O_2$/mg dry wt/hr) of heart, lung, kidney, liver, brain, thymus, and diaphragm was determined by the Warburg method. The tissue slices were taken from animals under basal metabolism regime (10–18 hr fasting). Respiration was measured in Krebs-Ringer phosphate solution (pH, 7.4) and in an atmosphere of oxygen. Higher Q_{O_2} values can be obtained in other media; but this was considered to be immaterial for the present purpose, which amounts to a comparison under standard conditions. There was no decline in the intensity of respiration during the experimental period (1 hr) except in brain tissue. The total number of determinations presented here is 253.

The order of the differences to be expected if the decrease of basal metabolic rate is based upon differences in tissue respiration can be estimated as follows. It appears that the surface rule holds intraspecifically for the rat (*9*); this is also stated by Kleiber (*10*), according to Benedict's data (*11*). Then

$$M = bW^{2/3}, \tag{1}$$

if M is the rate of basal metabolism, W the body weight, and b a constant; and correspondingly

$$\frac{M}{W} = bW^{-1/3} \tag{2}$$

for metabolic rate per unit weight. If, for example, the weights compared are 1 : 2 : 4 : 8 : 16, metabolic rates per unit weight (a measure of which is Q_{O_2}) should decrease in the ratio 1 : 0.79 : 0.63 : 0.50 : 0.4. Such differences should be easily detected by the Warburg method. We would not expect that the rate of decrease of Q_{O_2} with increasing size is the same in all tissues, but even then significant differences of the Q_{O_2} values should be found.

Actually, we did not find in our experiments systematic differences which would account for the decrease of the rate of total metabolism with increasing size (Table 1). A slight decrease in the average Q_{O_2} values is noticeable in some tissues (liver and heart) and especially in the diaphragm. But it is definitely smaller than the extent postulated by the surface rule, and not systematic with respect to the various tissues. Also if, instead of the surface rule, the 3/4 power of